BIOLOGICAL ACTIVITY OF 1α,24-DIHYDROXYCHOLECALCIFEROL; A NEW SYNTHETIC ANALOG OF THE HORMONAL FORM OF VITAMIN D

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1. Introduction

It is well established [1] that vitamin D_3 must be hydroxylated on C-25 in the liver and subsequently on C-1 in the kidney before it can carry out its functions in the intestine and bone. The resulting metabolite, 1α,25-dihydroxycholecalciferol (1α,25-$(OH)_2$-D_3)[2] is the presently known most potent and rapidly active analog of vitamin D_3 and is considered to be the hormonal form of the vitamin. Among many synthetic analogs, 1α-hydroxycholecalciferol (1α-OH-D_3) has the approximately equivalent activity of 1α,25-$(OH)_2$-D_3, in promoting intestinal calcium transport, bone mineral mobilization and curing rickets [2].

However, we have recently found [3] that 1α-OH-D_3 has a very high toxicity. LD_{50}-Values for 1α-OH-D_3 were 476 μg/kg (p.o.), 71 μg/kg (i.v.) in male mice and 440 μg/kg (p.o.), 56 μg/kg (i.v.) in female mice, respectively. Similar results were obtained in rats. In rats, oral administration of 2.5 μg/kg of 1α-OH-D_3 for 30 days was considered to be a nontoxic dose. The cause of death in both acute and subacute toxicity experiments might be due to hypercalcemia, considering the toxic signs.

In our continuing search for a less toxic vitamin D analog with enhanced and/or specific activity, we have now chosen 1α,24-dihydroxycholecalciferol (1α,24-$(OH)_2$-D_3 (fig.1) whose chemical synthesis has been described previously [4,5].

The present report describes the biological activity of 1α,24-$(OH)_2$-D_3 in normal and anephric rats. The activities of both stereoisomers of 1α,24-(OH_2)-D_3 are also compared with those of 1α-OH-D_3.

Abbreviations: 1α,25-$(OH)_2$-D_3 – 1α,25-dihydroxycholecalciferol, 1α,24-$(OH)_2$-D_3 – 1α,24-dihydroxycholecalciferol, 1α-OH-D_3 – 1α-hydroxycholecalciferol, 24-OH-D_3 – 24-hydroxycholecalciferol, 24,25-$(OH)_2$-D_3 – 24,25-dihydroxycholecalciferol, 25-OH-D_3 – 25-hydroxycholecalciferol, 1α,24,25-$(OH)_3$-D_3 – 1α,24,25-trihydroxycholecalciferol

Studies on 1α,24-dihydroxycholecalciferol, part I

2. Materials and methods

2.1. *Intestinal calcium transport and bone mobilization*

Weanling male wistar rats were fed for 4 weeks on a vitamin D-deficient low calcium diet (0.003%). At

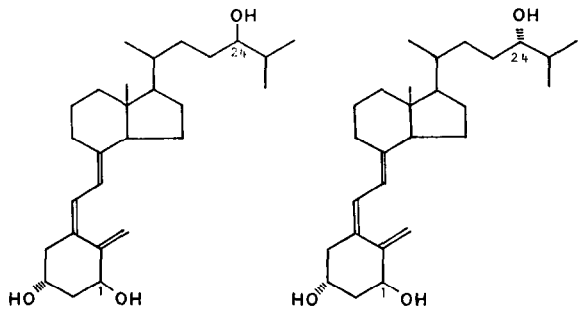


Fig.1. Structures of 1α,24-dihydroxycholecalciferol isomers.

the end of the 4th week, groups of 5 rats (weighing about 90 g), which were either intact or bilaterally nephrectomized, received intraperitoneally (in 95% ethanol) 1α-OH-D_3 or 1α,24-$(OH)_2$-D_3 (0.05 ml/100 g rat). The rats were sacrificed at various time from 4–24 h after drug administration, and intestinal calcium transport and serum calcium concentration were measured. Intestinal calcium-transport assay was performed as described by Martin and DeLuca [6]. Serum calcium concentration was determined by the OCPC (*o*-cresolphthalein comlexone) method [7].

2.2. *Antirachitic activity*

The antirachitic activity of the sterols was examined

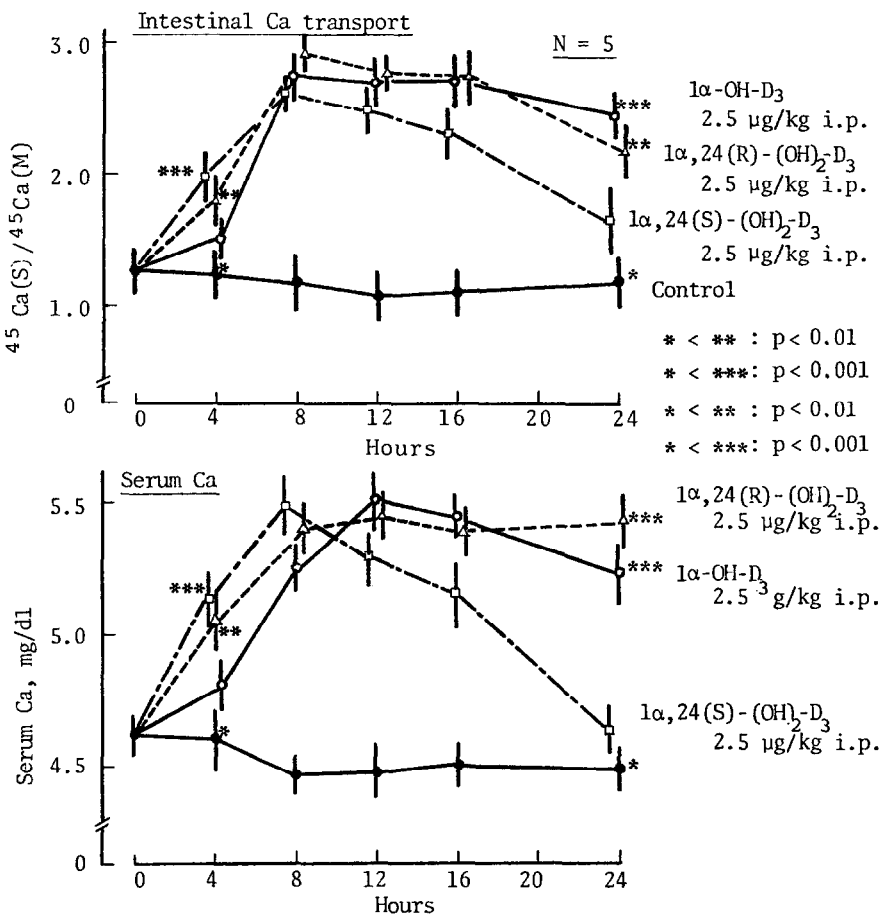


Fig.2. Effect of vitamin D_3 analogs on intestinal calcium transport and serum calcium level in vitamin D deficient rats. 2.5 μg/kg of the sterols dissolved in 95% EtOH was given intraperitoneally to the rats fed the low calcium, vitamin D-deficient diets for 4 weeks. At the indicated times, they were killed for determination of serum calcium concentration and intestinal calcium transport. There were 5 rats in each group. The vertical bars represent the SEM.

by means of the line-test method [8]. Weanling male Wistar rats were maintained in overhanging wire cages and fed on a rachitogenic diet (USP) for 3 weeks and were given either a single dose or a small daily dose of a vitamin D_3 analog for 6 days. Seven days after the first dose, the rats were killed and their radii (distal-end) and ulnae (proximal-end) were removed.

After cleaning of adhering tissues, each bone was cut with a clean sharp blade and a median, longitudinal section through the juncture of the epiphysis and diaphysis was made. The section was rinsed in purified water and immediately immersed in 1.5% silver nitrate solution for 1 min and rinsed again in purified water. The section was exposed, in water, to actinic light until the calcified areas develop a clearly defined stain. The new calcification epiphyseal plate width) was scored as described in US pharmacopeia using vitamin D_3 as a control [8].

3. Results

Both stereoisomers of 1α,24-$(OH)_2$-D_3 and 1α-OH-D_3 stimulate intestinal calcium transport and elicit a rise in serum calcium presumably at the expense of bone calcium (bone mineral mobilization) (fig.2). 1α,24-$(OH)_2$-D_3 was active 4 h after administration while 1α-OH-D_3 was inactive. After 8 h, each sterol caused maximum response and the effects were almost identical to each other. It is noted that

Table 1
Effect of vitamin D_3 analogs on intestinal calcium-transport and serum calcium in vitamin D-deficient rats

Compound	Dose (μg/kg)	Intestinal calcium-transport ^{45}Ca (S/M)	Serum calcium concentration (mg/dl)
Normal			
(95% Ethanol)		1.73 ± 0.08 (9)	4.8 ± 0.12 (9)
1α-OH-D_3	0.025 i.p.	1.74 ± 0.03 (5)	4.9 ± 0.10 (5)
	0.25 i.p.	2.15 ± 0.21 (5)	5.3 ± 0.25 (5)
	2.5 i.p.	2.72 ± 0.26 (5)[c]	5.5 ± 0.14 (5)[b]
1α,24-$(OH)_2$-D_3 (24 *R* Isomer)	0.025 i.p.	1.92 ± 0.08 (5)	5.3 ± 0.20 (5)[a]
	0.25 i.p.	2.83 ± 0.39 (5)[b]	5.5 ± 0.17 (5)[b]
	2.5 i.p.	3.53 ± 0.17 (5)[c]	5.9 ± 0.13 (5)[c]
1α,24-$(OH)_2$-D_3 (24 *S* Isomer)	0.025 i.p.	1.70 ± 0.03 (5)	4.6 ± 0.08 (5)
	0.25 i.p.	2.35 ± 0.17 (5)[b]	5.0 ± 0.17 (5)
	2.5 i.p.	2.58 ± 0.19 (5)[c]	5.5 ± 0.19 (5)[b]
Anephric			
(95% Ethanol)		1.34 ± 0.04 (4)	4.6 ± 0.09 (4)
1α-OH-D_3	2.5 i.p.	1.70 ± 0.06 (4)[b]	5.2 ± 0.12 (4)[b]
1α,24-$(OH)_2$-D_3 (24 *R* Isomer)	2.5 i.p.	1.76 ± 0.10 (4)[b]	5.4 ± 0.12 (4)[b]
1α,24-$(OH)_2$-D_3 (24 *S* Isomer)	2.5 i.p.	2.04 ± 0.18 (4)[b]	5.2 ± 0.08 (4)[b]

Student's t-test
[a] Significantly different from control, $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$

Rats, either intact or bilaterally nephrectomized, were intraperitoneally given the sterols and killed 8 h after administration for determination of serum calcium and intestinal calcium-transport. There were 4–9 rats in each groups (number in parentheses).

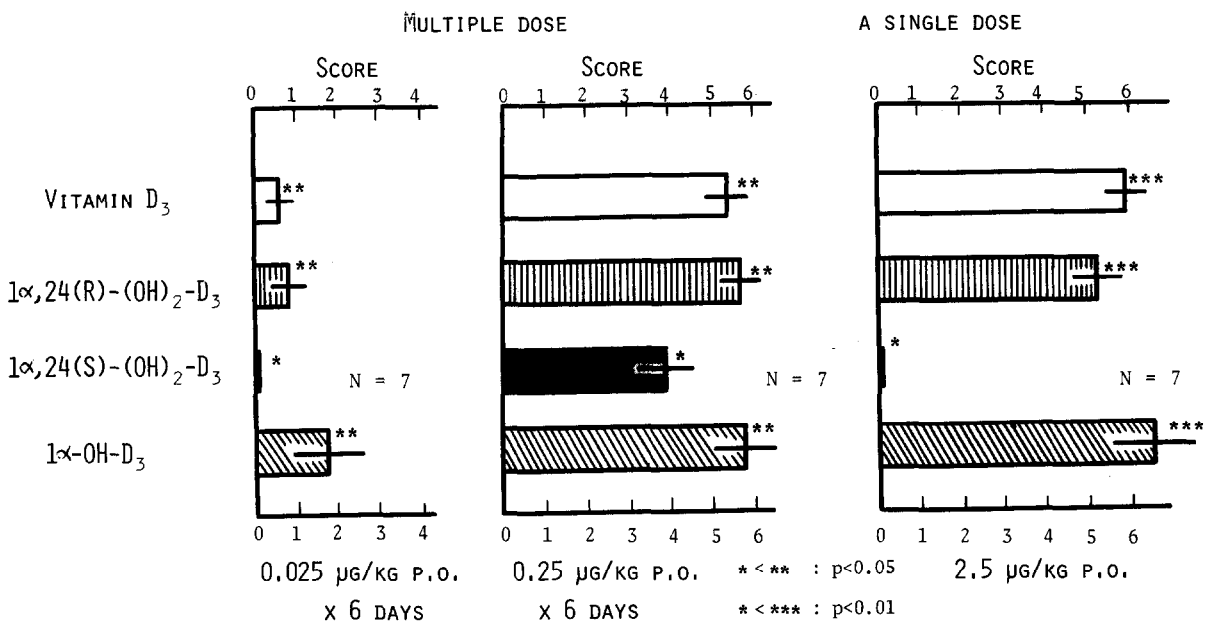
the S-isomer of 1α,24-$(OH)_2$-D_3 lost its effect 24 h after administration. This indicates that the *S*-isomer might be rapidly metabolized to an inactive form or excreted. 1α,24-$(OH)_2$-D_3 was also as active as 1α-OH-D_3 in anephric rats (table 1).

Fig.3 compares activity of 1α,24-$(OH)_2$-D_3 and 1α-OH-D_3 in curing rickets in rats. Both stereoisomers of 1α,24-$(OH)_2$-D_3 and 1α-OH-D_3 were active (fig.3). 1α-OH-D_3 was most active, given either as a single dose or small daily dose. The *R*-isomer of 1α,24-$(OH)_2$-D_3 was as active as 1α-OH-D_3, while the *S*-isomer was less active than vitamin D_3, and it did not show any activity when it had been given a single dose. This was consistent with the results mentioned above that the *S*-isomer of 1α,24$(OH)_2$-D_3 had lost its activity 24 h after administration.

These results revealed that 1α,24-DHCC was as active as 1α-OH-D_3 on intestine and bone both in intact and anephric rats and that the *S*-isomer had a short duration of action.

4. Discussion

Contrary to 1α-hydroxylation, the physiological meaning of 24-hydroxylation of vitamin D has not yet been clarified, although several factors are known to be involved [1]. 24-Hydroxycholecalciferol (24-OH-D_3) [9] and 24,25-dihydroxycholecalciferol (24,25-$(OH)_2$-D_3) [10] are as active as 25-hydroxycholecalciferol (25-OH-D_3) in stimulating intestinal calcium transport in vitamin D deficient rats. However, their 24 *S* stereoisomers have little or no activity in the mobilization of calcium from bone, in the elevation of serum phosphorus or in the calcification of bone, while the 24 *R* isomers are almost as active as 25-OH-D_3 in all of these systems [9,10]. Since these sterols were inactive both on intestine and bone in anephric rats [9,10] they presumably function after being hydroxylated on C-1. It has been also demonstrated that 1α,24,25-trihydroxycholecalciferol (1α,24,25-$(OH)_3$-D_3) has a preferential action on



ANTIRACHITIC ACTIVITY OF VITAMIN D ANALOGS IN RATS.

Fig.3. Antirachitic activity of vitamin D_3 analogs in rats. Weanling male Wistar rats were fed a rachitogenic diet for 3 weeks and were given either a single dose or a small daily dose of vitamin D_3 analogs. Seven days after the first dose, the rats were killed and their radii and ulnae were removed to score epiphyseal plate calcification [8]. There were 7 rats in each group. The horizontal bars represent the SEM.

intestine [11]. As above mentioned, both stereoisomers of 1α,24-$(OH)_2$-D_3 have about the same activity both on intestine and bone within 10 h after administration and after 24 h the *S*-isomer has lost activity. Possible explanations for these findings will be the subject of future experiments.

The presence report establishes that 1α,24-$(OH)_2$-D_3 is as potent as 1α-OH-D_3 in the stimulation of intestinal calcium transport and bone mineral mobilization both in normal and anephric rats. In view of these facts and its low toxicity (Kawashima, H. et al. in preparation) 1α,24-$(OH)_2$-D_3 is potentially atractive for clinical use in patients with renal failure and other metabolic bone diseases.

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